

# Neutralizing Antibody Responses against Autologous and Heterologous Viruses in Acute versus Chronic Human Immunodeficiency Virus (HIV) Infection: Evidence for a Constraint on the Ability of HIV To Completely Evade Neutralizing Antibody Responses

Steven G. Deeks,<sup>1\*</sup> Becky Schweighardt,<sup>2</sup> Terri Wrin,<sup>2</sup> Justin Galovich,<sup>2</sup> Rebecca Hoh,<sup>1</sup> Elizabeth Sinclair,<sup>1</sup> Peter Hunt,<sup>1</sup> Joseph M. McCune,<sup>1</sup> Jeffrey N. Martin,<sup>1</sup> Christos J. Petropoulos,<sup>2</sup> and Frederick M. Hecht<sup>1</sup>

*Department of Medicine, University of California at San Francisco, and San Francisco General Hospital, San Francisco, California,<sup>1</sup> and Monogram Biosciences, Inc., San Francisco, California<sup>2</sup>*

Received 13 January 2006/Accepted 18 February 2006

**Acute human immunodeficiency virus (HIV) infection is associated with the rapid development of neutralization escape mutations. The degree to which viral evolution persists in chronic infection has not been well characterized, nor is it clear if all patients develop high-level neutralization antibody escape. We therefore measured neutralizing antibody responses against autologous and heterologous viruses in a cohort of acutely and chronically infected subjects ( $n = 65$ ). Neutralizing antibody responses against both autologous virus and heterologous viruses were lower among individuals with acute infection than among those with chronic infection. Among chronically infected individuals, there was a negative correlation between the level of neutralizing antibodies against autologous virus and the level of viremia. In contrast, there was a positive correlation between the level of neutralizing antibodies against a panel of heterologous viruses and the level of viremia. Viral evolution, as defined by the presence of higher neutralizing titers directed against earlier viruses than against contemporaneous viruses, was evident for subjects with recent infection but absent for those with chronic infection. In summary, neutralizing antibody responses against contemporaneous autologous viruses are absent in early HIV infection but can be detected at low levels in chronic infection, particularly among those controlling HIV in the absence of therapy. HIV replication either directly or indirectly drives the production of increasing levels of antibodies that cross-neutralize heterologous primary isolates. Collectively, these observations indicate that although HIV continuously drives the production of neutralizing antibodies, there may be limits to the capacity of the virus to evolve continuously in response to these antibodies. These observations also suggest that the neutralizing antibody response may contribute to the long-term control of HIV in some patients while protecting against HIV superinfection in most patients.**

A major focus of the human immunodeficiency virus (HIV) vaccine effort is the development of broadly reacting neutralizing antibodies. An ideal antibody would retain potent anti-HIV activity against a diverse panel of primary isolates and would target conserved epitopes within the envelope (Env) protein that are fixed and unable to evolve in response to selective pressures. One manner in which to identify such antibodies (or to define whether such responses even occur) is to assess the role of neutralizing activity in the setting of established HIV infection. HIV-infected individuals may also provide access to plasma that retains potent and broad neutralizing antibody activities against heterologous viruses, including viruses that are prevalent in other HIV-infected individuals.

Most recently infected individuals mount a vigorous antibody response directed against autologous HIV. During this time, HIV typically evolves rapidly in response to this neutralizing antibody response. As a consequence, at any time during early HIV disease, antibody responses are more likely to recognize earlier autologous viruses than contemporaneous virus (2, 18, 36, 44, 51). The well-documented emergence of anti-

body escape during early HIV infection argues against a protective role of neutralizing antibodies in the setting of chronic infection. However, several issues remain unresolved. First, the degree to which neutralizing antibody escape evolution persists indefinitely has not been well defined. Theoretically, HIV may be constrained in its ability to continuously and fully escape neutralizing antibody responses over a period of years. Second, the rapid emergence of neutralizing escape mutations in the setting of primary HIV infection does not preclude the possibility that a small subset of patients may develop and maintain neutralizing antibody responses that effectively control HIV replication. Although some studies suggest that potent neutralizing antibody responses contribute to the control of HIV in patients with nonprogressive HIV infection (i.e., long-term nonprogressors) (9, 40, 41), other studies have failed to detect effective neutralizing responses in these patients (5, 20, 30). Finally, the presence of viral escape from neutralizing antibodies does not rule out the possibility that partially effective responses might persist. The latter concept is supported by recent observations indicating that residual antiretroviral drug pressure often persists in the presence of high-level drug resistance, suggesting that there are limits in the ability of HIV to completely evade some antiviral responses (4, 8, 13).

Antiretroviral therapy dramatically affects the complex rela-

\* Corresponding author. Mailing address: San Francisco General Hospital, 995 Potrero Avenue, San Francisco, CA 94110. Phone: (415) 476-4082, ext. 404. Fax: (415) 476-6953. E-mail: sdeeks@php.ucsf.edu.

tionship that exists between the virus and the host response. Although most studies have shown that anti-HIV neutralizing antibody responses decline after the introduction of therapy (presumably due to a decrease in antigenic stimulation), serial structured treatment interruptions, which are designed to enhance HIV-specific T-cell responses, have been associated with enhanced neutralizing activity against autologous virus (35). Similar observations have been reported among patients with intermittent viremia ("blips") (6). Finally, several studies have reported potent responses against autologous virus in the setting of partially effective antiretroviral therapy (6, 37, 38). The latter observations are consistent with a series of recent studies indicating that the emergence of drug-resistant HIV is associated with a decrease in relative virulence of HIV in vivo (12, 28) and that a significant subset of patients with low-level drug-resistant viremia exhibit heightened HIV-specific CD4<sup>+</sup> T-cell and CD8<sup>+</sup> T-cell responses (1, 14, 16, 42, 48).

Most earlier studies addressed the role of neutralizing antibody activity either by using lab-adapted strains (which could not provide insights about antibody responses against autologous virus) or by using labor-intensive approaches in which autologous virus was first cultured and passaged in vitro (which may have altered the virus). Because of these technical and practical limitations, novel techniques in which autologous virus can be assayed against patient-derived plasma in a high-throughput manner have been developed (11, 15, 44). In these techniques, pseudotyped vectors containing patient-derived *env* gene sequences undergo single rounds of replication in the presence or absence of patient-derived serum. These assays generally correlate well with the more established but labor-intensive peripheral blood mononuclear cell assays and have been recommended for use with studies assessing vaccine efficacy (7, 31, 34).

Given the number of unresolved questions regarding the role of neutralizing antibodies in the setting of established HIV infection and the recent development of assays able to quantify autologous responses in a high-throughput manner, we analyzed cross-sectional and longitudinal samples from a diverse group of recently and chronically infected adults. Our primary objective was to determine whether neutralizing activity directed against autologous virus has any associated impact on levels of viremia. We focused on four well-characterized groups of patients, including those with (i) acute infection, (ii) chronic infection with poorly controlled HIV replication (virologic noncontrollers), (iii) chronic infection with long-term control of HIV replication (virologic controllers), and (iv) chronic infection with long-term control of drug-resistant HIV (partial controllers on antiretroviral therapy [PCAT]). The latter two groups were chosen because evidence from our group and others indicates that they retain high levels of HIV-specific CD4<sup>+</sup> T cells, a subpopulation that is likely important for the generation and differentiation of a neutralizing antibody response (16). As a secondary objective, we analyzed the level of cross-neutralizing activity against a large panel of heterologous primary viruses.

#### MATERIALS AND METHODS

**Study design.** We assessed participants enrolled in two ongoing prospective cohort studies aimed at defining the immunologic and virologic characteristics associated with disease outcome in recently infected individuals (Options cohort)

or in chronically infected individuals (SCOPE cohort). All subjects in each cohort provided written informed consent.

Characteristics of the UCSF Options project have been described previously (21). Enrolled subjects must have evidence of acute or recent HIV infection as defined by (i) negative or weakly reactive HIV antibody test, enzyme immunoassay, and Western blot analysis results, with detectable plasma HIV RNA levels, (ii) documentation of a negative HIV antibody test result within the previous 12 months and a positive antibody test at screen, or (iii) a history compatible with recent HIV infection, with laboratory evidence of recent antibody seroconversion by use of a sensitive/less-sensitive enzyme immunoassay testing strategy (23). From this cohort, we selected individuals who presented with acute/recent HIV infection and who had plasma samples available prior to the introduction of antiretroviral therapy.

The UCSF SCOPE cohort has enrolled over 600 chronically infected adults. All subjects are observed every 4 months (14, 22). From this cohort, we selected two subsets of chronically infected individuals who were controlling HIV replication in the absence of fully effective antiretroviral therapy: (i) virologic controllers, defined as antiretroviral drug-untreated individuals who have a steady-state plasma HIV RNA level below 2,000 copies/ml, and (ii) PCAT, defined as antiretroviral drug-treated individuals with multidrug-resistant HIV and a steady-state plasma HIV RNA level between 500 and 10,000 copies/ml. As a comparator group, we selected a group of virologic noncontrollers, defined as antiretroviral drug-untreated individuals with plasma HIV RNA levels of >2,000 copies/ml.

Longitudinal analysis was performed with 32 subjects. This included five individuals with acute HIV infection who initiated and subsequently discontinued antiretroviral therapy. This interruption period was studied in detail, as this may mimic the period of untreated early HIV infection. We also studied chronically infected individuals who remained untreated (five controllers and three noncontrollers) or who remained on a stable partial suppressive regimen (14 PCAT subjects). Finally, we studied seven virologic noncontrollers who initiated and responded virologically to combination antiretroviral therapy. In each case, longitudinal neutralizing antibody responses were measured against the baseline autologous virus. Longitudinal neutralizing antibody responses against contemporaneous autologous viruses and against laboratory-adapted strains were also measured for most subjects.

**Cross-neutralizing activity against heterologous primary and laboratory viruses.** The capacity of patient-derived plasma to neutralize heterologous isolates from other members of the cohort was assessed using two separate matrices. Eight participants were included in the first experiment and 17 subjects in the second experiment. In each experiment, baseline plasma samples were tested against the baseline autologous virus from each of the other subjects. No subject contributed data to both experiments. For all subjects, we also tested the ability of plasma to neutralize heterologous laboratory stains (NL4-3 and JR-CSF), as outlined below.

**Neutralizing antibody assay.** The antibody neutralization assay is a modification of the Monogram Biosciences HIV coreceptor tropism assay (44). Briefly, HIV genomic RNA was isolated from virus stocks or plasma. DNA spanning the open reading frame of gp160 was amplified by reverse transcription PCR using forward and reverse primers located immediately upstream and downstream of the *env* initiation and termination codons, respectively. *env* PCR products were digested and ligated to compatible ends in an expression vector. These ligation products were then introduced into competent *Escherichia coli* cells by transformation, and plasmid DNA was purified from bacterial cultures. Virus particles containing patient virus Env proteins were produced by cotransfecting HEK293 cells with *env* libraries plus an *env* HIV genomic vector that contains a firefly luciferase indicator gene. Recombinant viruses pseudotyped with patient-derived virus Env proteins were harvested 48 h posttransfection and incubated for 1 h at 37°C with serial fourfold dilutions of heat-inactivated patient plasma samples. U87 cells that express CD4 plus the CCR5 and CXCR4 coreceptors were inoculated with virus-plasma dilutions. Virus infectivity was determined 72 h postinoculation by measuring the amount of luciferase activity expressed in infected cells. Neutralizing activity was displayed as the percent inhibition of viral replication (luciferase activity) at each antibody dilution compared to an antibody-negative control: % inhibition =  $[1 - (\text{luciferase with antibody} / \text{luciferase without antibody})] \times 100$ . Titers were calculated as the reciprocal of the plasma dilution conferring 50% inhibition.

Recombinant viruses pseudotyped with Env proteins from amphotropic murine leukemia virus (aMLV) were used as a negative control. These Env proteins are able to mediate virus entry in U87/CD4/CCR5/CXCR4 cells but are not inhibited by anti-HIV Env antibodies. Recombinant viruses pseudotyped with Env proteins from HIV laboratory isolates known to be relatively neutralization

TABLE 1. Cohort characteristics by treatment group

Characteristic	Median value (IQR) for group(s)				
	All ( <i>n</i> = 65)	Acute ( <i>n</i> = 24)	Controller ( <i>n</i> = 8)	Noncontroller ( <i>n</i> = 19)	PCAT ( <i>n</i> = 14)
Age (yr)	42 (37–48)	38 (32–46)	43 (41–48)	43 (38–46)	45 (41–50)
CD4 <sup>+</sup> T-cell count (cells/mm <sup>3</sup> )	506 (372–750)	544 (480–693)	799 (734–1,022)	374 (131–681)	417 (359–477)
CD8 <sup>+</sup> T-cell count (cells/mm <sup>3</sup> )	1,111 (738–1,315)	1,140 (1,002–1,274)	1,319 (888–1,475)	1,025 (650–1,299)	1,071 (763–1,284)
Plasma HIV-1 RNA level (log <sub>10</sub> copies/ml)	3.94 (3.18–4.81)	4.77 (4.30–5.39)	2.93 (2.45–3.06)	4.42 (3.92–4.85)	2.75 (2.55–3.41)
Nadir CD4 <sup>+</sup> T-cell count (cells/mm <sup>3</sup> ) <sup>a</sup>					110 (65–222)
Duration of HIV infection (yr)	5.4 (0.2–13.1)	0.2 (0.2–0.2)	12.6 (7.8–14.7)	8.7 (4.1–13.2)	12.7 (8.9–14.9)

<sup>a</sup> Nadir CD4<sup>+</sup> T-cell count was defined as the lowest prior CD4<sup>+</sup> T-cell count and was measured only for the antiretroviral drug-treated cohort.

sensitive (e.g., NL4-3 and SF162) or neutralization resistant (e.g., JR-CSF) were also studied in each experiment.

For samples derived from antiretroviral drug-treated patients, modifications to this assay were needed to exclude the potential inhibitory activity of drugs in patient-derived serum. HIV expression vectors were therefore constructed with multidrug-resistant reverse transcriptase and protease in experiments involving antiretroviral drug-treated patients. All pseudotyped virions (e.g., NL4-3, JR-CSF, aMLV, and autologous virus) contained the same reverse transcriptase/protease in these studies.

**T-cell activation.** T-cell activation (as defined by the coexpression of CD38 and HLA-DR) was measured for a subset of chronically infected individuals. As previously described (22), these studies were performed using freshly collected, EDTA-anticoagulated whole blood and analyzed by four-color flow cytometry with a Beckman Coulter Epics XL flow cytometer. Activated T cells were identified using fluorescein isothiocyanate-conjugated anti-HLA-DR, phycoerythrin-conjugated anti-CD38, phycoerythrin-cyanin red 5.1-conjugated anti-CD3, and phycoerythrin-Texas Red-conjugated anti-CD4 or -CD8. To exclude monocytes and natural killer cells, expression of CD3 was included in the definition of both CD4<sup>+</sup> and CD8<sup>+</sup> T cells. Cell subpopulations expressing the activation markers CD38 and/or HLA-DR were gated from the total CD3<sup>+</sup> CD4<sup>+</sup> or CD3<sup>+</sup> CD8<sup>+</sup> populations on a two-dimensional dot plot where quadrant gates, defined by pertinent isotype control antibodies, were used to delineate positive and negative populations.

**Statistical analysis.** Nonparametric tests were used for all analyses. Differences in continuous variables between patient groups were analyzed with the Mann-Whitney U test. All correlations were determined using Spearman's rank correlation. Changes in neutralizing antibody titers were assessed over time by using generalized estimating equations, treating the month of observation as a categorical predictor and log transforming the neutralizing antibody titer to meet model assumptions. Changes in neutralizing antibody titers over time were assessed with nonlinear tests of trend.

## RESULTS

**Subject characteristics.** A total of 65 individuals were included in this study (Table 1). Twenty-four subjects had evidence of recent HIV infection (median estimated duration of infection, 2.4 months; interquartile range [IQR], 2.2 to 2.4). Eight subjects were virologic controllers and had maintained low-level viremia in the absence of therapy. Nineteen subjects were virologic noncontrollers. Fourteen subjects had maintained partial control of viral replication on antiretroviral therapy despite the presence of drug-resistant HIV.

**Neutralizing responses against autologous virus.** The degrees to which antibodies found in patient plasma neutralize autologous virus from the same time point were assessed at the first time point for all patients. Low-level responses with titers in the range of 1:20 to 1:100 were observed with most chronically infected subjects (Fig. 1A). When all chronically infected subjects were considered, there was a consistently higher response to the contemporaneous autologous virus than to the

non-HIV envelope control virus (murine leukemia virus [MLV]), suggesting that low-level autologous activity may exist in these individuals (*P* of <0.001 for the response to autologous virus versus MLV). In contrast, a neutralizing antibody response against autologous virus was generally absent in individuals with acute HIV infection and was much lower than that observed in individuals with chronic infection (*P* < 0.001) (Fig. 1A).

There was a negative correlation between the neutralizing titer against autologous virus and the plasma HIV RNA level among all subjects ( $\rho = -0.29$ , *P* = 0.03, *n* = 57) but no association between the neutralizing antibody response against autologous virus and the CD4<sup>+</sup> T-cell count. Since this analysis may have been confounded by rapidly changing HIV RNA levels in the setting of acute infection as well as by the complicated impact which treatment may have had on virus-host interactions, we analyzed untreated chronically infected subjects separately. A negative association between the neutralizing antibody response against autologous virus and the steady-state level of viremia was also observed within this subset ( $\rho = -0.40$ , *P* = 0.05) (Fig. 1B). Collectively, these observations provide indirect evidence supporting a persistent and partially effective neutralizing antibody response against autologous virus.

**Cross-neutralizing activity against heterologous viruses.** The capacity of antibodies in patient-derived plasma to neutralize primary isolates from other members of the cohort was assessed using two separate matrices. In the first experiment, plasma samples from five virologic controllers and three virologic noncontrollers were tested against the baseline virus from each of these subjects (Table 2). In the second experiment, plasma samples from three virologic controllers, eight virologic noncontrollers, and six PCAT were tested against the baseline virus from each of these subjects (Tables 3 and 4).

The magnitude of the cross-neutralizing response was defined based on the average neutralizing titer against all heterologous viruses. The average magnitude of the neutralizing antibody against heterologous viruses was consistently higher than the response observed against contemporaneous autologous virus (*P* = 0.02; Wilcoxon rank test). This trend was observed within each of the three subsets of subjects studied: virologic controllers, virologic noncontrollers, and PCAT.

The breadth of the cross-neutralizing response was defined based on the number of viruses that were effectively neutralized by any given patient-derived plasma sample (where a

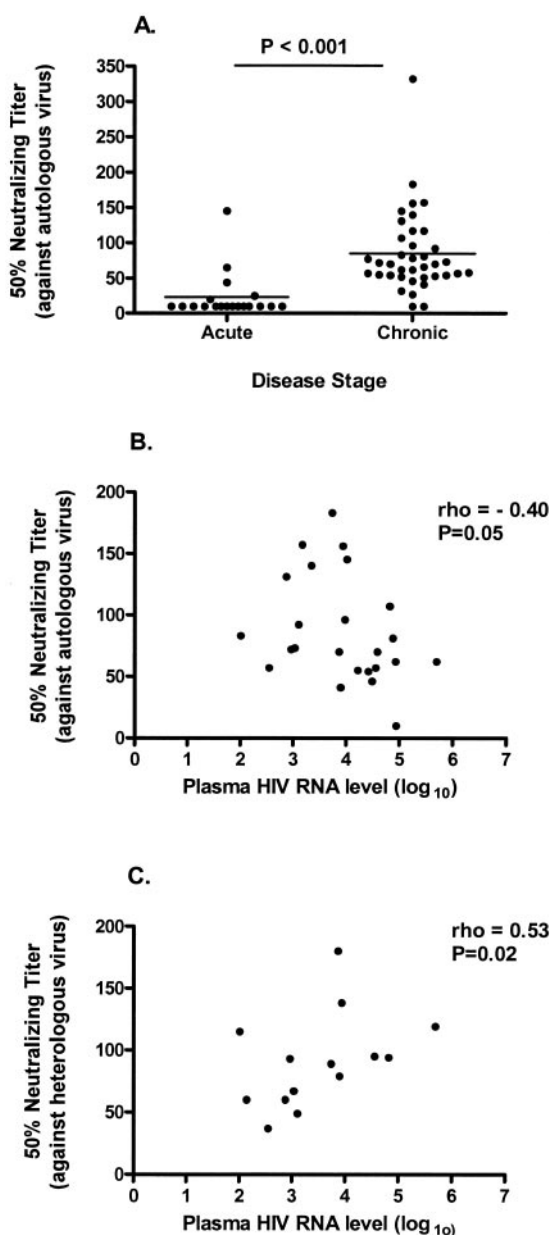


FIG. 1. (A) Neutralizing antibody titers against contemporaneous autologous virus for subjects with acute HIV infection ( $n = 19$ ) and for subjects with chronic infection ( $n = 41$ ). (B) Relationship between HIV RNA levels and the neutralizing titers against autologous virus for antiretroviral drug-untreated, chronically infected subjects. There was a negative correlation between viremia and the autologous neutralizing antibody titer ( $\rho = -0.40$ ,  $P = 0.05$ ). (C) Relationship between HIV RNA levels and the mean neutralizing titers against a panel of viruses obtained from other antiretroviral drug-untreated subjects with chronic infection. There was a positive correlation between viremia and the average heterologous response ( $\rho = 0.53$ ,  $P = 0.02$ ). For each panel, the neutralizing antibody titer is defined as the reciprocal of the dilution of plasma that produces 50% inhibition of target cell infection (higher titers signify greater levels of neutralizing activity).

positive response was defined by the presence of a  $>3$ -fold-higher response against the heterologous virus than against the aMLV control). The breadths of the responses varied but were

generally higher for virologic noncontrollers than for virologic controllers: plasma from the noncontrollers neutralized a median of 71% of other viruses (IQR, 48% to 88%), while plasma from the controllers neutralized a median of 43% of other viruses (IQR, 25% to 54%). The PCAT subjects had very low levels of cross-neutralizing activity (plasma from these individuals neutralized a median of 4% of other viruses [IQR, 0% to 19%]).

When all subjects were considered, there was a positive association between the magnitude of the neutralizing responses against heterologous virus and the level of viremia ( $\rho = 0.48$ ,  $P = 0.01$ ,  $n = 25$ ). This effect was also seen when we limited our analysis to the chronically untreated subset ( $\rho = 0.53$ ,  $P = 0.02$ ,  $n = 19$ ) (Fig. 1C). There was also a similar association between the breadth of the neutralizing response against heterologous virus (in terms of the proportion of viruses which tested positive) and the level of viremia ( $\rho = 0.50$ ,  $P = 0.01$ ,  $n = 25$ ).

The degree to which antibodies in patient plasma samples could neutralize heterologous viruses was also assessed by measuring responses to three well-characterized laboratory strains (NL4-3, SF162, and JR-CSF). Neutralizing responses to these viruses were much higher than those observed to occur against contemporaneous autologous virus ( $P$  of  $<0.001$  for each pairwise comparison). Also, as seen with autologous responses, the response to these viruses was much higher for patients with chronic infection than for patients with acute

TABLE 2. Cross-neutralizing activity against heterologous virus, determined by using plasma from antiretroviral-untreated subjects<sup>a</sup>

Source of virus	Neutralizing antibody titer from plasma from <sup>b</sup> :							
	Virologic controller patient					Virologic noncontroller patient		
	1009	1050	1056	1086	4006	1027	1028	1503
Patient groups and ID no.								
Controllers								
1009	68*	42	70	725*	50	433*	122*	41
1050	54	<b>136*</b>	83*	491*	76*	245*	167*	153*
1056	19	36	72	50	46	31	42	58
1086	35	27	26	<b>73*</b>	52	34	299*	55
4006	47	65*	58	104*	<b>126*</b>	68*	59	102*
Noncontrollers								
1027	21	34	42	71*	42	<b>246*</b>	78*	69
1028	65*	88*	101*	300*	76*	270*	<b>98*</b>	95*
1503	48	91*	77	457*	75*	395*	128*	<b>186*</b>
Virus and lab strains								
aMLV	20	20	27	20	23	20	20	26
JR-CSF	32	59	63	174	62	155	184	57
NL4-3	818	1,630	877	1,519	524	1,864	2,204	2,848

<sup>a</sup> Neutralizing HIV antibody titers were measured in plasma derived from chronically infected, antiretroviral-untreated subjects meeting our study definition of virologic controllers or noncontrollers (patient identification [ID] numbers are given). These data reflect the average cross-neutralization titers determined by using plasma from two to four consecutive visits.

<sup>b</sup> The neutralizing antibody titer is defined as the reciprocal of the dilution of plasma that produces 50% inhibition of target cell infection. Values marked with an asterisk indicate neutralizing titers that are at least three times greater than those observed against the negative control virus (aMLV). Numbers in bold indicate that the neutralizing antibody titer was derived using plasma and virus from the same subject (autologous virus). Titers against laboratory strains (JR-CSF and NL4-3) and against aMLV are also shown.

TABLE 3. Cross-neutralizing activity against heterologous virus, determined by using plasma from antiretroviral-untreated subjects<sup>a</sup>

Source of virus	Neutralizing antibody titer from plasma from <sup>b</sup> :										
	Virologic controller patient			Virologic noncontroller patient							
	1504	1508	1516 <sup>c</sup>	1002	1006	1008	1017	1048	1512	1515	3018
Patient groups and ID no.											
Controllers											
1504	<b>72</b>	82	45	196*	375*	264*	117*	212*	397*	57	101
1508	88*	<b>83</b>	83*	118*	130*	118*	107*	105*	498*	33	103
Noncontrollers											
1002	51	67	37	<b>94*</b>	118*	37	51	80*	211*	46	
1006	57	99	71*	158*	<b>57</b>	177*	100*	143*	251*	30	50
1008	123*	100	64*	117*	395*	<b>52</b>	68*	114*	192*	33	92
1017	244*	175*	64*	388*	137*	175*	<b>105*</b>	1,119*	175*	425*	114
1048	93*	92	35	131*	350*	64*	95*	<b>102*</b>	573*	50	86
1512	57	56	32	114*	49	55	42	97*	<b>140</b>	33	77
1515	91*	249*	69*	367*	122*	89*	57	310*	615*	<b>41</b>	139
3018	103*	140	81*	249*	266*	187*	153*	198*	1,272*	77*	<b>107</b>
PCAT											
2004	39	89	47	56	47	48	48	79*	1,195*	37	81
3002	61	82	58	98*	165*	75*	40	152*	245*	38	78
3037	129*	156	95*	418*	820*	154*	176*	373*	864*	118*	81
3039	56	54	26	229*	153*	55	41	83*	144	43	95
Virus and lab strains											
JR-CSF	102	116	75	390	546	182	117	613	314	42	108
NL4-3	4,713	1,720	906	1,891	2,058	639	3,702	5,043	1,950	1,366	781
aMLV	24	58	20	31	26	20	20	22	50	20	65

<sup>a</sup> Neutralizing HIV antibody titers were measured in plasma derived from chronically infected, antiretroviral drug-untreated subjects meeting our study definition of virologic controllers or noncontrollers (patient identification [ID] numbers are given). Neutralization titers were measured against a panel of heterologous primary viruses representing all three subject groups (virologic controllers, virologic noncontrollers, and PCAT).

<sup>b</sup> See footnote *b* of Table 2 for explanations of neutralizing antibody titer, asterisks, and boldface type.

<sup>c</sup> Virus could not be amplified for subject 1516.

infection ( $P$  of  $<0.001$  for each pairwise comparison of acute versus chronic infection).

#### Viral evolution is evident in recent but not chronic infection.

Previous studies have shown that among recently infected patients, viral evolution in response to neutralizing antibody activity is so rapid that titers against autologous virus obtained at the concurrent time point are low to undetectable, while titers against virus sampled several months earlier are often high (44, 51). Here, we assessed the degree to which neutralizing antibody titers against autologous virus emerged over time for the five acutely infected subjects who initiated and responded virologically to a combination regimen and who subsequently discontinued therapy (Fig. 2A). The neutralizing antibody response against autologous virus and laboratory strains was low prior to the introduction of therapy. Interruption of therapy was associated with a variable increase in HIV RNA levels but a consistent increase in the ability of patient-derived plasma to neutralize NL4-3, SF162, and JR-CSF ( $P$  of  $<0.001$  for NL4-3,  $P$  of  $<0.0001$  for SF162, and  $P$  of 0.03 for JR-CSF; test-of-trend analysis). Autologous responses against the baseline virus also increased over time ( $P = 0.002$ ; test-of-trend analysis). Of note, although the response to contemporaneous virus was low for most subjects, it increased with time, suggesting residual activity of these responses at later time points ( $P = 0.04$ ; test-of-trend analysis). These data among acutely infected individuals interrupting effective antiretroviral therapy are consistent with previous observations made in the setting of un-

treated primary infection (44) and support the concept that HIV is able to evolve rapidly in response to active antibody-mediated immunologic pressure. However, the observation that the response directed against contemporaneous virus increases with time suggests that the ability of the virus to completely evade neutralizing responses may be limited.

We also studied longitudinal changes in the setting of chronic infection (Fig. 2B). A total of 23 individuals were studied (five virologic controllers, four virologic noncontrollers, and 14 partial controllers on antiretroviral therapy). In each case, the response to the baseline virus was assessed every 16 weeks over a period of 1 year (where baseline was defined as the first sample available for this analysis). In contrast to observations with early HIV disease, neutralizing activity against baseline autologous virus did not increase over time ( $P > 0.30$ ; test-of-trend analysis). Responses to laboratory strains also remained stable over time ( $P$  of  $>0.30$  for each virus). Hence, viral evolution was not detected with patients who had been infected for several years or more.

**Antiretroviral therapy rapidly decreases the level of neutralizing antibodies.** Seven antiretroviral drug-untreated individuals initiated and responded to a combination antiretroviral regimen (Fig. 3, top). Neutralizing titers against autologous virus were low for these virologic noncontrollers prior to the introduction of antiretroviral therapy and did not change significantly during the first 1 to 2 years of treatment (Fig. 3, middle). In contrast, neutralizing activity against NL4-3 was

TABLE 4. Cross-neutralizing activity against heterologous virus, determined by using plasma from antiretroviral-treated subjects with partial viral suppression<sup>a</sup>

Source of virus	Neutralizing antibody titer from plasma from PCAT patient <sup>b</sup> :					
	2004	3002	3025 <sup>c</sup>	3037	3039	3049 <sup>c</sup>
<b>Patient groups and ID no.</b>						
<b>Controllers</b>						
1504	10	115*	234	58	211	72
1508	10	102*	237	53	425*	60
1002	10	72*				
<b>Noncontrollers</b>						
1006	21	127*	207	33	173	59
1008	21	259*	238	45	163	74
1017	36	241*	250	91	225	141*
1048	40	237*	226	40	176	102*
1512	10	36	258	33	148	43
1515	21	89*	246	44	186	89*
3018	25	165*	240	39	163	73
<b>PCAT</b>						
2004	<b>10</b>	43	236	23	177	45
3002	26	<b>54</b>	252	37	358	51
3037	10	197*	249	<b>56</b>	159	61
3039	10	50	172	29	<b>163</b>	53
<b>Virus and lab strains</b>						
JR-CSF	34	437	227	41	215	84
NL4-3	260	1,802	347	435	986	1,191
aMLV	20	21	175	41	131	29

<sup>a</sup> Neutralizing HIV antibody titers were measured in plasma derived from chronically infected, antiretroviral drug-treated subjects meeting our study definition of PCAT (patient identification [ID] numbers are given). Neutralization titers were measured against the same panel of heterologous primary viruses outlined in footnote *a* of Table 3.

<sup>b</sup> See footnote *b* of Table 2 for explanations of neutralizing antibody titer, asterisks, and boldface type.

<sup>c</sup> Virus could not be amplified for subjects 3025 and 3049.

high prior to therapy and declined dramatically within the first 4 months of observation (Fig. 3, bottom). Two patients experienced intermittent viremia during therapy. These isolated periods of detectable viremia were temporally associated with transient increases in the response against autologous virus but not against NL4-3 (Fig. 3, middle). These observations indicate that HIV antigen drives the production of neutralizing antibodies in the setting of advanced HIV disease. The rapid decrease in NL4-3 responses, with no change in autologous responses, provides further evidence that most of the anti-HIV specific neutralizing activity in these chronically infected individuals is ineffective.

**Immune activation and heterologous responses.** Several mechanisms may account for the observation that neutralizing responses against heterologous viruses, including antigen-driven stimulation of ineffective HIV-specific antibodies (i.e., "original antigenic sin") and/or nonspecific immune activation, increased during chronic HIV infection. Although we did not measure B-cell function directly in this study, we did study the proportions of CD4<sup>+</sup> and CD8<sup>+</sup> T cells coexpressing both CD38 and HLA-DR. Among chronically infected individuals, there was a consistent significant direct association between CD4<sup>+</sup> T-cell activation and the neutralizing antibody response

against a panel of heterologous viruses ( $\rho = 0.52$ ,  $P = 0.01$ ,  $n = 22$ ) and between CD4<sup>+</sup> T-cell activation and neutralizing antibody response against NL4-3 ( $\rho = 0.36$ ,  $P = 0.36$ ,  $n = 37$ ). There were similar associations with CD8<sup>+</sup> T-cell activation ( $\rho$  of 0.42 and  $P$  of 0.05 for responses to heterologous primary viruses and  $\rho$  of 0.31 and  $P$  of 0.06 for responses to NL4-3).

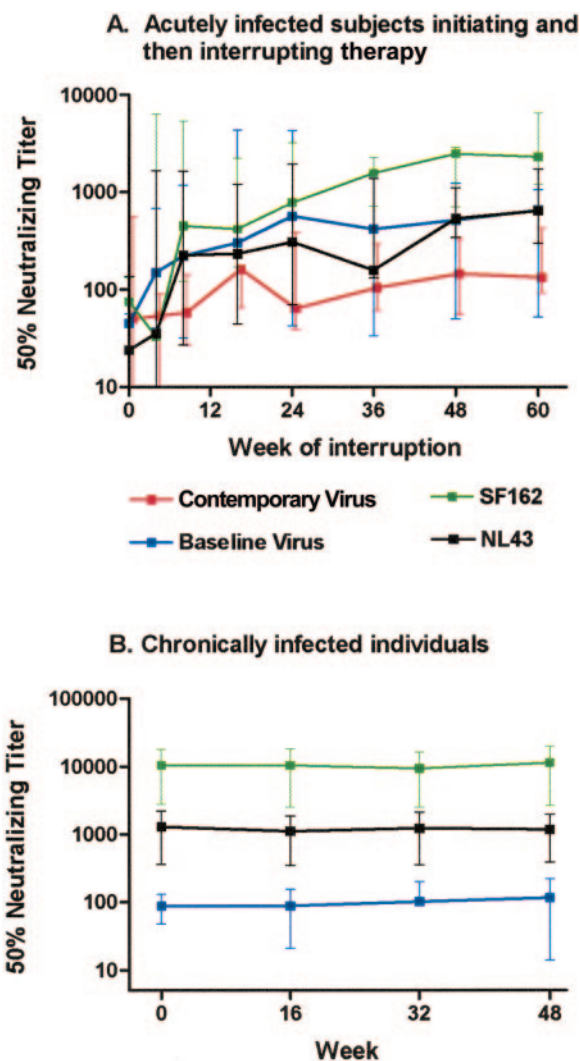


FIG. 2. Change in response to baseline autologous virus and to laboratory viruses over time in recent (A) and chronic (B) HIV disease. (A) Five acutely infected subjects initiated and later interrupted antiretroviral therapy. Viral rebound was associated with increased responses against contemporaneous virus ( $P = 0.04$ ), the baseline autologous virus ( $P = 0.002$ ), SF162 ( $P < 0.001$ ), and NL4-3 ( $P < 0.001$ ). The response to earlier autologous viruses was consistently higher than the response to autologous virus (data not shown). (B) The neutralizing responses to baseline autologous viruses was consistently higher than the response to autologous virus (data not shown). There was no evidence for a change over time ( $P$  of  $>0.30$  for each virus). Changes in neutralizing antibody titers were analyzed using generalized estimating equations and assessed with nonlinear tests of trend. Median values and interquartile ranges are shown.

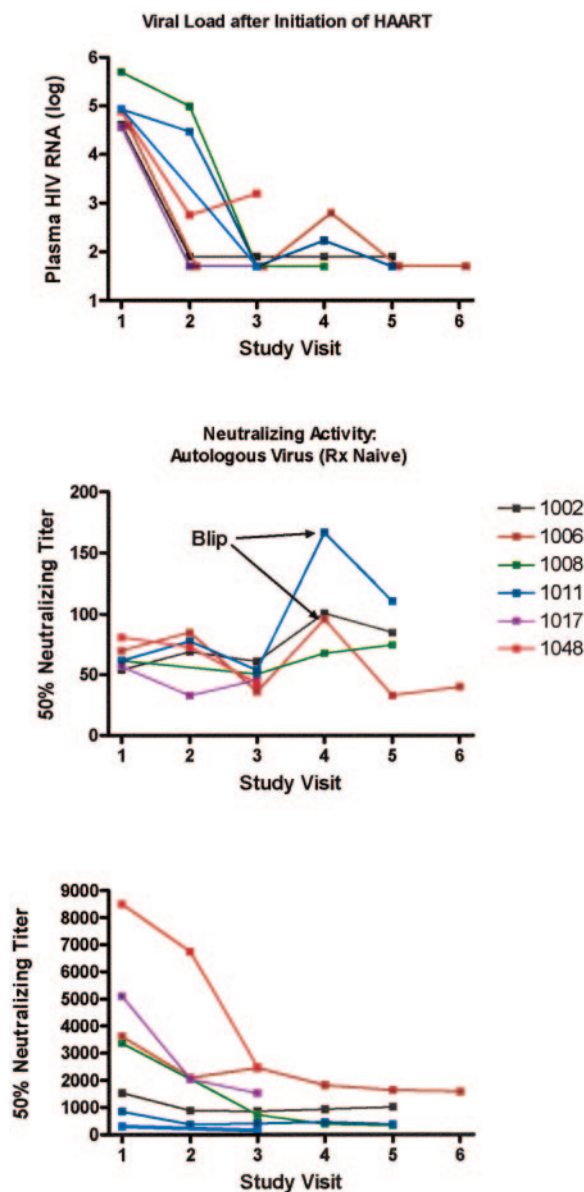


FIG. 3. Change in response to autologous virus and NL4-3 after initiation of an effective antiretroviral treatment regimen. (Top) Seven antiretroviral drug-untreated subjects with chronic HIV disease initiated and responded virologically to combination antiretroviral therapy. (Middle) Neutralizing titers against autologous virus were low and did not change significantly during the first 1 to 2 years of treatment. Two subjects experienced intermittent viremia ("blips") upon therapy; autologous responses appeared to increase concurrently with these episodes. (Bottom) In contrast, neutralizing activity against NL4-3 was high prior to therapy and decreased rapidly during treatment. HAART, highly active antiretroviral therapy. Study visit 1 refers to the visit just prior to the initiation of antiretroviral therapy. The subsequent study visits were each separated by 4 months. One subject (3022; light blue) did not have evaluable responses directed against autologous virus.

## DISCUSSION

Using a single-cycle recombinant virus assay to measure neutralizing antibodies against autologous and heterologous primary isolates, we made a number of observations relevant to

the role of neutralizing antibodies in the settings of acute and chronic HIV infection. First, neutralizing activity against contemporaneous autologous virus was generally low, particularly with acutely infected individuals. However, among chronically infected individuals, there was a negative correlation between the response to autologous virus and plasma HIV RNA levels, suggesting that these antibodies may contribute to the control of HIV replication. Second, although the neutralizing antibody response against autologous virus was low during early HIV infection, it did increase with time, again suggesting that the ability of HIV to fully evade neutralizing antibodies is limited. Third, viral evolution (as defined by the ability of plasma to neutralize earlier virus more effectively than contemporaneous virus) was evident in early but not in chronic HIV infection. Fourth, many subjects, particularly those with advanced disease and higher levels of viremia, had high cross-neutralizing antibody responses directed against laboratory viruses and moderate cross-neutralizing titers directed against heterologous primary isolates. Finally, the initiation of highly active antiretroviral therapy resulted in a rapid decrease in the anti-NL4-3 neutralizing titer, with levels approaching a new steady state within 4 months of starting therapy. Collectively, these observations indicate that although HIV evolution is rapid in early HIV disease it does not persist indefinitely and that there may be constraints on the virus's ability to fully evade neutralizing antibody responses. Regardless of whether these neutralizing antibodies exert an anti-HIV effect in chronic disease, they appear to retain the ability to cross-neutralize viruses from other individuals, which may have clinical implications, as discussed below.

One of the central questions we sought to address is whether neutralizing antibodies determine, in part, the steady-state level of viral replication in the setting of either treated or untreated chronic HIV infection. Based on data indicating that an effective antigen-specific CD4<sup>+</sup> T-cell response is critical for maintaining an effective B-cell response (10, 32), we selected from our cohort a subset of subjects who were known to have high-level CD4<sup>+</sup> T-cell responses (i.e., virologic controllers and PCAT) (14). Although high-level neutralizing titers directed against contemporaneous autologous virus were not observed with most of these subjects, our observation that the autologous response was higher for patients with lower levels of viremia argues for a protective role (at least in the setting of chronic untreated HIV disease). Given the cross-sectional nature of this analysis, it is difficult to conclude whether detectable autologous neutralizing antibody responses are a cause or a consequence of low levels of viral replication. However, our observation that heterologous virus response increases with viremia argues against a mechanism whereby antibody production is generally spared in patients naturally controlling viral replication.

The well-documented ability of HIV to escape from neutralizing antibody responses is often cited as strong evidence against any protective role for these antibodies in chronic disease. In our current study, we extend these findings to include acutely infected individuals who initiated therapy at the time of their diagnosis and who subsequently interrupted antiretroviral therapy (hypothetically, the interruption phase may provide insights into host-virus dynamics during acute disease). As seen with untreated primary HIV disease (18, 36, 44, 51), neutral-

izing antibody responses against contemporaneous autologous viruses were generally lower than the responses against earlier viruses, suggesting that the immune system was not able to efficiently respond to a rapidly evolving quasi-species. There may be limits, however, to the ability of the virus to fully escape, as evidenced by the observation that responses to contemporaneous virus increased over time.

Although neutralizing activity against autologous virus was generally low in the setting of chronic infection, neutralizing activity against heterologous virus was often high. This was particularly true with regard to the response to NL4-3 and SF162 for chronically infected individuals with high-level viremia. NL4-3 is a lab-adapted strain that has presumably lost those virologic factors that protect against antibody-mediated neutralization *in vivo* (47). NL4-3-specific responses may therefore be a surrogate for the overall level of HIV-specific neutralizing titers. Our observation that neutralization titers to NL4-3 increased with advanced disease and then decreased rapidly in response to antiretroviral drug-mediated suppression of viral replication argues that the generation of these antibodies in the setting of advanced immunodeficiency is driven by antigenic stimulation. HIV-mediated production of antibodies may reflect nonspecific B-cell activation and/or an "original antigenic sin" phenomenon. With regard to the former point, our group and others have shown high levels of T-cell activation for patients with advanced disease and have determined that these levels decrease dramatically in response to treatment (22, 29, 50). Chronic polyclonal B-cell activation has also been reported in the setting of advanced HIV disease (27, 37, 45). Although we did not measure B-cell function directly in this study, we observed a consistent relationship between both CD4<sup>+</sup> and CD8<sup>+</sup> T-cell activation (as defined by expression of CD38 and HLA-DR) and the level of neutralizing antibody responses against heterologous virus, suggesting that immune activation drives some of the HIV-specific neutralizing antibodies in the setting of chronic disease. With regard to the second point ("original antigenic sin"), it is possible that B cells and other antibody-secreting cells that were primed by earlier viral strains continue to produce antibody in response to antigen-driven stimulation from related but neutralization-resistant strains. This phenomenon has been well described for other chronic viral infections (17, 25, 33).

Low to moderate cross-neutralizing activity was also observed when plasma from a disparate group of chronically infected subjects was tested against a panel of patient-derived viruses. Although the clinical relevance of these observations remains unclear, it is interesting to speculate that such neutralizing antibody responses against heterologous viruses might protect against HIV reinfection (or superinfection). Most well-documented cases of HIV superinfection have occurred in the setting of recent HIV infection (3, 24, 26, 43, 46, 52). Superinfection appears to be very uncommon in the setting of advanced HIV disease (19, 49). This potential relationship between the duration of HIV disease and susceptibility to HIV superinfection was tested empirically in a macaque model, where it was shown that the risk of superinfection was high during the acute stage but waned rapidly with time (39). Our observations that higher HIV RNA levels in chronic disease are associated with increased titers of response against heterologous viruses (in terms of both magnitude and breadth)

suggest that humoral immunity might protect against reinfection in some individuals.

Our study has several limitations that deserve comment. Most importantly, our observation that the level of viremia is negatively correlated with the autologous responses is based on a cross-sectional analysis, making it difficult to discern cause and effect. Although low-level viremia may be a consequence of effective HIV immunity, it is also possible that low-level viremia may preserve immune function and the production of antibodies that have an insignificant impact on viral replication. This seems to be less likely given the observation that viral replication appears to drive the production of HIV-specific neutralizing antibodies, as shown by the consistent association between HIV RNA levels and responses to heterologous viruses, as well as by the observation that antiretroviral treatment decreases these responses. A second limitation pertains to our observations regarding viral evolution, which was evident for acutely infected individuals interrupting therapy but not evident for chronically infected individuals in steady state. A more appropriate comparison group for our chronically infected individuals would have been recently infected patients who did not receive antiretroviral therapy. However, a previous study of recently infected individuals using the same methods provided consistent evidence for viral evolution in early untreated HIV disease (44). More importantly, it should be emphasized that our conclusion regarding the lack of viral evolution is based on indirect inferences (i.e., a lack of increasing neutralizing titers against earlier viruses). A more definitive manner to test this issue is to sequence virus from plasma and peripheral blood mononuclear cells over time and then to directly compare rates of viral escape in early versus late HIV infection. Finally, although our conclusions suggest that HIV may not be able to fully evolve to escape all neutralizing pressures, it is also possible that the host may have limits in its capacity to continue to generate novel epitope-specific antibody responses.

In conclusion, low but measurable neutralizing antibody responses against autologous virus exist in some chronically infected individuals, suggesting a role of these responses in the durable control of HIV replication. Viral evolution, as defined based on evolving neutralizing responses over time, is clearly evident with recent infection but not with long-term chronic infection. The latter observation suggests that HIV might be constrained in its ability to evade neutralizing response indefinitely and/or that the immune system is eventually exhausted and unable to generate highly effective neutralizing antibody responses over time. Finally, chronic HIV infection drives the ongoing production of HIV-specific antibodies that effectively neutralize heterologous viruses. Whether this observation reflects antigen-driven expansion and/or abnormal B-cell activation remains to be determined.

#### ACKNOWLEDGMENTS

This work was supported in part by grants from NIAID (AI052745, AI055273, AI47062, and AI44595), the California AIDS Research Center (CC99-SF, ID01-SF-049), the National Institutes of Health UCSF/Gladstone Institute of Virology & Immunology Center for AIDS Research (P30 MH59037, P30 AI27763), the Center for AIDS Prevention Studies (P30 MH62246), and the General Clinical Research Center at San Francisco General Hospital (5-MO1-RR00083-37).

## REFERENCES

- Alatrakchi, N., C. Duvivier, D. Costagliola, A. Samri, A. Marcelin, G. Kamkamidze, M. Astriti, R. Agher, V. Calvez, B. Autran, and C. Katlama. 2005. Persistent low viral load on antiretroviral therapy is associated with T cell-mediated control of HIV replication. *AIDS* **19**:25–33.
- Albert, J., B. Abrahamsson, K. Nagy, E. Aurelius, H. Gaines, G. Nystrom, and E. M. Fenyo. 1990. Rapid development of isolate-specific neutralizing antibodies after primary HIV-1 infection and consequent emergence of virus variants which resist neutralization by autologous sera. *AIDS* **4**:107–112.
- Altfeld, M., T. M. Allen, X. G. Yu, M. N. Johnston, D. Agrawal, B. T. Korber, D. C. Montefiori, D. H. O'Connor, B. T. Davis, P. K. Lee, E. L. Maier, J. Harlow, P. J. Goulder, C. Brander, E. S. Rosenberg, and B. D. Walker. 2002. HIV-1 superinfection despite broad CD8<sup>+</sup> T-cell responses containing replication of the primary virus. *Nature* **420**:434–439.
- Barbour, J. D., T. Wrin, R. M. Grant, J. N. Martin, M. R. Segal, C. J. Petropoulos, and S. G. Deeks. 2002. Evolution of phenotypic drug susceptibility and viral replication capacity during long-term virologic failure of protease inhibitor therapy in human immunodeficiency virus-infected adults. *J. Virol.* **76**:11104–11112.
- Barker, E., C. E. Mackewicz, G. Reyes-Teran, A. Sato, S. A. Stranford, S. H. Fujimura, C. Christopherson, S. Y. Chang, and J. A. Levy. 1998. Virological and immunological features of long-term human immunodeficiency virus-infected individuals who have remained asymptomatic compared with those who have progressed to acquired immunodeficiency syndrome. *Blood* **92**:3105–3114.
- Binley, J. M., A. Trkola, T. Ketas, D. Schiller, B. Clas, S. Little, D. Richman, A. Hurley, M. Markowitz, and J. P. Moore. 2000. The effect of highly active antiretroviral therapy on binding and neutralizing antibody responses to human immunodeficiency virus type 1 infection. *J. Infect. Dis.* **182**:945–949.
- Binley, J. M., T. Wrin, B. Korber, M. B. Zwick, M. Wang, C. Chappey, G. Stiegler, R. Kunert, S. Zolla-Pazner, H. Katinger, C. J. Petropoulos, and D. R. Burton. 2004. Comprehensive cross-clade neutralization analysis of a panel of anti-human immunodeficiency virus type 1 monoclonal antibodies. *J. Virol.* **78**:13232–13252.
- Campbell, T. B., N. S. Shulman, S. C. Johnson, A. R. Zolopa, R. K. Young, L. Bushman, C. V. Fletcher, E. R. Lanier, T. C. Merigan, and D. R. Kuritzkes. 2005. Antiviral activity of lamivudine in salvage therapy for multidrug-resistant HIV-1 infection. *Clin. Infect. Dis.* **41**:236–242.
- Carotenuto, P., D. Looij, L. Keldermans, F. de Wolf, and J. Goudsmit. 1998. Neutralizing antibodies are positively associated with CD4<sup>+</sup> T-cell counts and T-cell function in long-term AIDS-free infection. *AIDS* **12**:1591–1600.
- Chirmule, N., N. Oyaizu, V. S. Kalyanaraman, and S. Pahwa. 1992. Inhibition of normal B-cell function by human immunodeficiency virus envelope glycoprotein, gp120. *Blood* **79**:1245–1254.
- Decker, J. M., F. Bibollet-Ruche, X. Wei, S. Wang, D. N. Levy, W. Wang, E. Delaporte, M. Peeters, C. A. Derdeyn, S. Allen, E. Hunter, M. S. Saag, J. A. Hoxie, B. H. Hahn, P. D. Kwong, J. E. Robinson, and G. M. Shaw. 2005. Antigenic conservation and immunogenicity of the HIV coreceptor binding site. *J. Exp. Med.* **201**:1407–1419.
- Deeks, S. G., R. Hoh, R. M. Grant, T. Wrin, J. D. Barbour, A. Narvaez, D. Cesar, K. Abe, M. B. Hanley, N. S. Hellmann, C. J. Petropoulos, J. M. McCune, and M. K. Hellerstein. 2002. CD4<sup>+</sup> T cell kinetics and activation in human immunodeficiency virus-infected patients who remain viremic despite long-term treatment with protease inhibitor-based therapy. *J. Infect. Dis.* **185**:315–323.
- Deeks, S. G., R. Hoh, T. B. Neillands, T. Liegler, F. Aweeka, C. J. Petropoulos, R. M. Grant, and J. N. Martin. 2005. Interruption of treatment with individual therapeutic drug classes in adults with multidrug-resistant HIV-1 infection. *J. Infect. Dis.* **192**:1537–1544.
- Deeks, S. G., J. N. Martin, E. Sinclair, J. Harris, T. B. Neillands, H. T. Maecker, E. Hagos, T. Wrin, C. J. Petropoulos, B. Brecht, and J. M. McCune. 2004. Strong cell-mediated immune responses are associated with the maintenance of low-level viremia in antiretroviral-treated individuals with drug-resistant human immunodeficiency virus type 1. *J. Infect. Dis.* **189**:312–321.
- Derdeyn, C. A., J. M. Decker, F. Bibollet-Ruche, J. L. Mokili, M. Muldoon, S. A. Denham, M. L. Heil, F. Kasolo, R. Musonda, B. H. Hahn, G. M. Shaw, B. T. Korber, S. Allen, and E. Hunter. 2004. Envelope-constrained neutralization-sensitive HIV-1 after heterosexual transmission. *Science* **303**:2019–2022.
- Emu, B., E. Sinclair, D. Favre, W. J. Moretto, P. Hsue, R. Hoh, J. N. Martin, D. F. Nixon, J. M. McCune, and S. G. Deeks. 2005. Phenotypic, functional, and kinetic parameters associated with apparent T-cell control of human immunodeficiency virus replication in individuals with and without antiretroviral treatment. *J. Virol.* **79**:14169–14178.
- Fazekas de St. Groth, S., and R. G. Webster. 1966. Disquisitions on original antigenic sin. I. Evidence in man. *J. Exp. Med.* **124**:331–345.
- Geffin, R., C. Hutto, C. Andrew, and G. B. Scott. 2003. A longitudinal assessment of autologous neutralizing antibodies in children perinatally infected with human immunodeficiency virus type 1. *Virology* **310**:207–215.
- Gonzales, M. J., E. Delwart, S. Y. Rhee, R. Tsui, A. R. Zolopa, J. Taylor, and R. W. Shafer. 2003. Lack of detectable human immunodeficiency virus type 1 superinfection during 1072 person-years of observation. *J. Infect. Dis.* **188**:397–405.
- Harrer, T., E. Harrer, S. A. Kalams, T. Elbeik, S. I. Staprans, M. B. Feinberg, Y. Cao, D. D. Ho, T. Yilma, A. M. Caliendo, R. P. Johnson, S. P. Buchbinder, and B. D. Walker. 1996. Strong cytotoxic T cell and weak neutralizing antibody responses in a subset of persons with stable nonprogressing HIV type 1 infection. *AIDS Res. Hum. Retrovir.* **12**:585–592.
- Hecht, F. M., M. P. Busch, B. Rawal, M. Webb, E. Rosenberg, M. Swanson, M. Chesney, J. Anderson, J. Levy, and J. O. Kahn. 2002. Use of laboratory tests and clinical symptoms for identification of primary HIV infection. *AIDS* **16**:1119–1129.
- Hunt, P. W., J. N. Martin, E. Sinclair, B. Brecht, E. Hagos, H. Lampiris, and S. G. Deeks. 2003. T cell activation is associated with lower CD4<sup>+</sup> T cell gains in human immunodeficiency virus-infected patients with sustained viral suppression during antiretroviral therapy. *J. Infect. Dis.* **187**:1534–1543.
- Janssen, R. S., G. A. Satten, S. L. Stramer, B. D. Rawal, T. R. O'Brien, B. J. Weiblen, F. M. Hecht, N. Jack, F. R. Cleghorn, J. O. Kahn, M. A. Chesney, and M. P. Busch. 1998. New testing strategy to detect early HIV-1 infection for use in incidence estimates and for clinical and prevention purposes. *JAMA* **280**:42–48.
- Jost, S., M. C. Bernard, L. Kaiser, S. Yerly, B. Hirschel, A. Samri, B. Autran, L. E. Goh, and L. Perrin. 2002. A patient with HIV-1 superinfection. *N. Engl. J. Med.* **347**:731–736.
- Klennerman, P., and R. M. Zinkernagel. 1998. Original antigenic sin impairs cytotoxic T lymphocyte responses to viruses bearing variant epitopes. *Nature* **394**:482–485.
- Koelsch, K. K., D. M. Smith, S. J. Little, C. C. Ignacio, T. R. Macaranas, A. J. Brown, C. J. Petropoulos, D. D. Richman, and J. K. Wong. 2003. Clade B HIV-1 superinfection with wild-type virus after primary infection with drug-resistant clade B virus. *AIDS* **17**:F11–F16.
- Lane, H. C., H. Masur, L. C. Edgar, G. Whalen, A. H. Rook, and A. S. Fauci. 1983. Abnormalities of B-cell activation and immunoregulation in patients with the acquired immunodeficiency syndrome. *N. Engl. J. Med.* **309**:453–458.
- Ledergerber, B., J. D. Lundgren, A. S. Walker, C. Sabin, A. Justice, P. Reiss, C. Mussini, F. Wit, A. d'Arminio Monforte, R. Weber, G. Fusco, S. Staszewski, M. Law, R. Hogg, F. Lampe, M. J. Gill, F. Castelli, and A. N. Phillips. 2004. Predictors of trend in CD4-positive T-cell count and mortality among HIV-1-infected individuals with virological failure to all three antiretroviral-drug classes. *Lancet* **364**:51–62.
- Liu, Z., W. G. Cumberland, L. E. Hultin, H. E. Prince, R. Detels, and J. V. Giorgi. 1997. Elevated CD38 antigen expression on CD8<sup>+</sup> T cells is a stronger marker for the risk of chronic HIV disease progression to AIDS and death in the Multicenter AIDS Cohort Study than CD4<sup>+</sup> cell count, soluble immune activation markers, or combinations of HLA-DR and CD38 expression. *J. Acquir. Immune Defic. Syndr. Hum. Retrovir.* **16**:83–92.
- Loomis-Price, L. D., J. H. Cox, J. R. Mascola, T. C. VanCott, N. L. Michael, T. R. Fouts, R. R. Redfield, M. L. Robb, B. Wahren, H. W. Sheppard, and D. L. Bix. 1998. Correlation between humoral responses to human immunodeficiency virus type 1 envelope and disease progression in early-stage infection. *J. Infect. Dis.* **178**:1306–1316.
- Mascola, J. R., P. D'Souza, P. Gilbert, B. H. Hahn, N. L. Haigwood, L. Morris, C. J. Petropoulos, V. R. Polonis, M. Sarzotti, and D. C. Montefiori. 2005. Recommendations for the design and use of standard virus panels to assess neutralizing antibody responses elicited by candidate human immunodeficiency virus type 1 vaccines. *J. Virol.* **79**:10103–10107.
- Moir, S., K. M. Ogwaro, A. Malaspina, J. Vasquez, E. T. Donoghue, C. W. Hallahan, S. Liu, L. A. Ehler, M. A. Planta, S. Kottilli, T. W. Chun, and A. S. Fauci. 2003. Perturbations in B cell responsiveness to CD4<sup>+</sup> T cell help in HIV-infected individuals. *Proc. Natl. Acad. Sci. USA* **100**:6057–6062.
- Mongkolsapaya, J., W. Dejnirattisai, X. N. Xu, S. Vasanawathana, N. Tangthawornchaikul, A. Chairunsri, S. Sawasdivorn, T. Duangchinda, T. Dong, S. Rowland-Jones, P. T. Yenchitsomanus, A. McMichael, P. Malasit, and G. Screaton. 2003. Original antigenic sin and apoptosis in the pathogenesis of dengue hemorrhagic fever. *Nat. Med.* **9**:921–927.
- Montefiori, D. 2003. HIV vaccine development: immunological and biological challenges. Presented at the Keystone Symposia, Banff, Alberta, Canada, 29 March to 4 April 2003.
- Montefiori, D. C., T. S. Hill, H. T. Vo, B. D. Walker, and E. S. Rosenberg. 2001. Neutralizing antibodies associated with viremia control in a subset of individuals after treatment of acute human immunodeficiency virus type 1 infection. *J. Virol.* **75**:10200–10207.
- Moog, C., H. J. Fleury, I. Pellegrin, A. Kirn, and A. M. Aubertin. 1997. Autologous and heterologous neutralizing antibody responses following initial seroconversion in human immunodeficiency virus type 1-infected individuals. *J. Virol.* **71**:3734–3741.
- Morris, L., J. M. Binley, B. A. Clas, S. Bonhoeffer, T. P. Astill, R. Kost, A. Hurley, Y. Cao, M. Markowitz, D. D. Ho, and J. P. Moore. 1998. HIV-1 antigen-specific and -nonspecific B cell responses are sensitive to combination antiretroviral therapy. *J. Exp. Med.* **188**:233–245.
- Morris, M. K., D. A. Katzenstein, D. Israelski, A. Zolopa, R. M. Hendry, and C. V. Hanson. 2001. Characterization of the HIV-1 specific humoral immune

- response during highly active antiretroviral therapy (HAART). *J. Acquir. Immune Defic. Syndr.* **28**:405–415.
39. Otten, R. A., D. L. Ellenberger, D. R. Adams, C. A. Fridlund, E. Jackson, D. Pieniazek, and M. A. Rayfield. 1999. Identification of a window period for susceptibility to dual infection with two distinct human immunodeficiency virus type 2 isolates in a *Macaca nemestrina* (pig-tailed macaque) model. *J. Infect. Dis.* **180**:673–684.
  40. Pantaleo, G., S. Menzo, M. Vaccarezza, C. Graziosi, O. J. Cohen, J. F. Demarest, D. Montefiori, J. M. Orenstein, C. Fox, L. K. Schrager, et al. 1995. Studies in subjects with long-term nonprogressive human immunodeficiency virus infection. *N. Engl. J. Med.* **332**:209–216.
  41. Pilgrim, A. K., G. Pantaleo, O. J. Cohen, L. M. Fink, J. Y. Zhou, J. T. Zhou, D. P. Bolognesi, A. S. Fauci, and D. C. Montefiori. 1997. Neutralizing antibody responses to human immunodeficiency virus type 1 in primary infection and long-term-nonprogressive infection. *J. Infect. Dis.* **176**:924–932.
  42. Price, D. A., G. Scullard, A. Oxenius, R. Braganza, S. A. Beddows, S. Kazmi, J. R. Clarke, G. E. Johnson, J. N. Weber, and R. E. Phillips. 2003. Discordant outcomes following failure of antiretroviral therapy are associated with substantial differences in human immunodeficiency virus-specific cellular immunity. *J. Virol.* **77**:6041–6049.
  43. Ramos, A., D. J. Hu, L. Nguyen, K. O. Phan, S. Vanichseni, N. Promadej, K. Choopanya, M. Callahan, N. L. Young, J. McNicholl, T. D. Mastro, T. M. Folks, and S. Subbarao. 2002. Intersubtype human immunodeficiency virus type 1 superinfection following seroconversion to primary infection in two injection drug users. *J. Virol.* **76**:7444–7452.
  44. Richman, D. D., T. Wrin, S. J. Little, and C. J. Petropoulos. 2003. Rapid evolution of the neutralizing antibody response to HIV type 1 infection. *Proc. Natl. Acad. Sci. USA* **100**:4144–4149.
  45. Shirai, A., M. Cosentino, S. F. Leitman-Klinman, and D. M. Klinman. 1992. Human immunodeficiency virus infection induces both polyclonal and virus-specific B cell activation. *J. Clin. Invest.* **89**:561–566.
  46. Smith, D. M., J. K. Wong, G. K. Hightower, C. C. Ignacio, K. K. Koelsch, E. S. Daar, D. D. Richman, and S. J. Little. 2004. Incidence of HIV superinfection following primary infection. *JAMA* **292**:1177–1178.
  47. Sullivan, N., Y. Sun, J. Li, W. Hoffmann, and J. Sodroski. 1995. Replicative function and neutralization sensitivity of envelope glycoproteins from primary and T-cell line-passaged human immunodeficiency virus type 1 isolates. *J. Virol.* **69**:4413–4422.
  48. Tenorio, A. R., K. Y. Smith, D. R. Kuritzkes, B. E. Sha, B. Donoval, R. Young, C. Jennings, J. Bremer, S. Shott, A. Landay, and H. A. Kessler. 2003. HIV-1-infected antiretroviral-treated patients with prolonged partial viral suppression: clinical, virologic, and immunologic course. *J. Acquir. Immune Defic. Syndr.* **34**:491–496.
  49. Tsui, R., B. L. Herring, J. D. Barbour, R. M. Grant, P. Bacchetti, A. Kral, B. R. Edlin, and E. L. Delwart. 2004. Human immunodeficiency virus type 1 superinfection was not detected following 215 years of injection drug user exposure. *J. Virol.* **78**:94–103.
  50. Valdez, H., E. Connick, K. Y. Smith, M. M. Lederman, R. J. Bosch, R. S. Kim, M. St. Clair, D. R. Kuritzkes, H. Kessler, L. Fox, M. Blanchard-Vargas, and A. Landay. 2002. Limited immune restoration after 3 years' suppression of HIV-1 replication in patients with moderately advanced disease. *AIDS* **16**:1859–1866.
  51. Wei, X., J. M. Decker, S. Wang, H. Hui, J. C. Kappes, X. Wu, J. F. Salazar-Gonzalez, M. G. Salazar, J. M. Kilby, M. S. Saag, N. L. Komarova, M. A. Nowak, B. H. Hahn, P. D. Kwong, and G. M. Shaw. 2003. Antibody neutralization and escape by HIV-1. *Nature* **422**:307–312.
  52. Yang, O. O., E. S. Daar, B. D. Jamieson, A. Balamurugan, D. M. Smith, J. A. Pitt, C. J. Petropoulos, D. D. Richman, S. J. Little, and A. J. Brown. 2005. Human immunodeficiency virus type 1 clade B superinfection: evidence for differential immune containment of distinct clade B strains. *J. Virol.* **79**:860–868.